

## Serotypes of Group B Streptococci and Their Relation to Hyaluronidase Production and Hydrolysis of Salicin

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A total of 252 strains of group B streptococci were serotyped and examined for their ability to ferment lactose (lac+), to hydrolyze salicin (sal+), and to produce hyaluronidase (hy+). Of these strains, 67 had been isolated from bacteremia and meningitis in infants less than 2 months old. Eighty-one strains were isolated from bacteremia and meningitis in adults, and 104 strains were from various other infections. Type III was the most common in neonatal disease, especially if isolates from cases of bacteremia in infants less than 10 days of age were not included. Only 6% of the strains were lac+. Sal+/hy+ strains were never type III, but 91% of the strains belonging to the other serotypes were sal+/hy+. Results showed that 81% of the sal+/hy- strains and 95% of the sal-/hy+ strains were type III, and sal-/hy+ strains were more than twice as frequent as sal+/hy- strains in serious neonatal infections, in contrast to the other two disease groups, in which the opposite was found to be the case. These reactions may be used as additional markers in epidemiological studies.

The incidence of neonatal bacteremia and meningitis due to group B streptococci has been estimated to have increased to 3 cases per 1,000 live births in the United States (1). In Denmark, the number of cases, although in 1977 it had increased significantly during the last 10 years, was considerably lower, i.e., from 0.2 to at most 0.3 cases per 1,000 live births (8). It is known that certain serotypes, namely, types Ia and III, predominate (2, 8, 10). The reason why neonatal group B infections have become more frequent remains obscure.

In an attempt to further characterize group B streptococci isolated from serious neonatal infections, we have examined their hyaluronidase production because this enzyme might conceivably be of importance for virulence. We also chose to study fermentation of lactose and hydrolysis of salicin because of the potential usefulness of these characteristics in differentiation, as indicated by earlier studies (3, 5, 6, 9). Strains isolated from cases of bacteremia and meningitis in adults, as well as noninvasive strains isolated from various sites in patients not seriously infected, were included in the study for comparative purposes.

### MATERIALS AND METHODS

**Bacterial strains.** (i) Sixty-seven strains were isolated from blood or cerebrospinal fluid of children less than 2 months of age; 23 of the 33 strains isolated from blood were from children less than 10 days old. (ii) Seventy-seven strains were isolated from blood, and 4 were from cerebrospinal fluid of adults. (iii) Finally, 104 strains were isolated from various sources such as

the urogenital system, the respiratory tract, rectum, and wounds of patients with neither bacteremia nor meningitis.

**Serotyping.** Serotyping of group B streptococci was carried out as described previously (9) using Lancefield extracts and unabsorbed type antisera against the polysaccharide type antigens Ia, Ib, II, and III in a capillary precipitation test. The typing antisera were without any demonstrable content of anti-group B antibodies. We did not examine for the presence of the protein antigens Ibc, R, and X.

**Production of hyaluronidase.** Strains were grown in Todd-Hewitt broth for 24 h at 37°C. After centrifugation, the supernatant was diluted with acetate buffer (pH 6.0) and incubated at 37°C with an equal volume of a solution of streptococcal hyaluronic acid (7) in acetate buffer (pH 6.0). Strains were registered as producing hyaluronidase if the hyaluronic acid content of the reaction mixture (0.2 mg/ml) had been degraded by the supernatants diluted at least 1:10 after incubation for 1 h, as measured turbidimetrically (4). (It was found that if a supernatant did not degrade hyaluronic acid in dilution 1:10, the undiluted supernatant did not either.)

**Fermentation of lactose and hydrolysis of salicin.** Fermentation of lactose and hydrolysis of salicin were studied using phenol red broth base (Difco Laboratories) supplemented with beef extract (Difco) to a final concentration of 0.2% and with lactose or salicin to a concentration of 1.0%. Test tubes were inoculated with bacteria taken from a blood agar culture plate, incubated at 37°C, and observed for 8 days.

### RESULTS

**Serotypes.** Type III was more common and type II was more seldom in serious neonatal disease than in the two other disease groups (50

versus 30% and 10 versus 20%, respectively [Table 1]). The type distribution among the 23 strains isolated from the blood of infants under 10 days of age, however, was different from that of the other isolates from neonatal disease (early-onset meningitis and late-onset bacteremia and meningitis), in that type III was less frequent and type II was more common. If these 23 strains are excluded from the isolates from infants, type III alone accounts for 70% and types Ib and II each account for 2% only.

**Biochemical investigations.** With the exception of fermentation of lactose, the results of biochemical investigations are shown in Table 2. The percentage of lactose-positive strains was low in all three disease groups, altogether 6% (16 of 252), and the strains were randomly distributed among serotypes and disease groups. Seventy-five percent (190 of 252) of all strains produced hyaluronidase (hy+), and this did not vary among the three disease groups of strains, but only 57% of the type III strains were hy+. Of the strains, 84% (212 of 252) were salicin positive (sal+). Salicin-negative (sal-) strains were all hy+, and 95% (38 of 40) of such strains (sal-/hy+) belonged to serotype III. Of the sal+ strains, 74% were also hy+; such strains (sal+/hy+) were never type III, although they were by far the most common (91%) among the other serotypes. However, 84% (50 of 62) of the sal+/hy- strains were type III.

Only the combinations sal+/hy- and sal-/hy+ varied in frequency among the three disease groups. Among neonatal disease isolates, sal-/hy+ was more than twice as common as sal+/hy-, whereas among isolates of the other two groups the reverse was found.

Neither hydrolysis of salicin nor production of hyaluronidase varied among neonatal isolates (early onset/late onset; bacteremia/meningitis) in a way that could not be explained by the

TABLE 2. *Biotypes and serotypes of group B streptococci*

Patients	Bio-type <sup>a</sup>		Serotype <sup>b</sup>				
	sal	hy	Ia	Ib	II	III	NT <sup>c</sup>
Bacteremic and meningitic infants (<2 months old)	+	+	16	4	6	0	3
	+	-	2	0	0	10	2
	-	+	0	0	0	24	0
Bacteremic and meningitic adults	+	+	18	11	14	0	6
	+	-	0	0	1	18	3
	-	+	1	0	0	8	1
Infections other than bacteremia or meningitis	+	+	34	9	20	0	9
	+	-	1	0	1	22	2
	-	+	0	0	0	6	0

<sup>a</sup> sal, Salicin; hy, hyaluronidase; all salicin-negative strains (sal-) produced hyaluronidase, i.e., they were hy+.

<sup>b</sup> Each value indicates number of strains.

<sup>c</sup> NT, Nontypable.

distribution of the serotypes, to which these reactions are correlated.

## DISCUSSION

The serotype distribution among neonatal disease isolates of group B streptococci found in the present study compares well with that reported before (2, 8-10). Type III was the most common type, especially among isolates from early-onset meningitis and from late-onset meningitis and bacteremia. There was a more even distribution between types Ia, Ib, II, and III among isolates from early-onset bacteremia and from various infections in adults including bacteremia and meningitis.

Between 75% and 95% of group B streptococci isolated from humans have previously been reported to be unable to ferment lactose, in contrast to the predominantly lactose-positive group B strains of bovine origin (3, 5). Only 6% of our strains fermented lactose.

Lack of hydrolysis of salicin was almost exclusively seen among type III strains.

Production of hyaluronidase among group B streptococci has not been studied systematically before. Although 75% of the strains examined did produce hyaluronidase, the finding that 57% of the type III strains did not produce it indicates that the virulence of group B strains is not dependent on production of hyaluronidase. However, there were more than twice as many hyaluronidase-producing type III strains among isolates from neonatal infections than among

TABLE 1. *Serotype distribution among group B streptococci*

Source <sup>a</sup>	No. of strains	% with the following serotypes <sup>b</sup> :				
		Ia	Ib	II	III	NT <sup>c</sup>
CSF or blood (children)	67	27	6	9	51	7
CSF or blood (adults)	81	23	14	19	32	12
Various other sources	104	34	9	20	27	11

<sup>a</sup> See text for further specifications. CSF, Cerebrospinal fluid.

<sup>b</sup> Each value indicates percentage of the total number of strains from each source.

<sup>c</sup> NT, Nontypable.

isolates from noninvasive infections in adults. Therefore, further studies of this property should be undertaken.

Hydrolysis of salicin and production of hyaluronidase appear to be useful characteristics to include in future studies of the epidemiology of group B disease as a supplement to serotyping.

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